CLAIMS

1. A novel transferase which acts on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three glucose residues from the reducing end are α-1,4-linked, so as to transfer the first α-1,4 linkage from the reducing end into an α-1,α-1 linkage.

2. A novel transferase which acts on a maltooligosaccharide, all the glucose residues of the maltooligosaccharide being α-1,4-linked, so as to transfer the first α-1,4 linkage from the reducing end into an α-1,α-1 linkage.

3. The novel transferase claimed in Claim 1 or 2, wherein its molecular weight measured by SDS-polyacrylamide gel electrophoresis is 74,000 to 76,000, approximately.

4. The novel transferase claimed in any one of Claims 1 to 3, wherein the transferase has the following physical and chemical properties:

(1) Optimum pH with in the range from 4.5 to 6.0;

(2) Optimum temperature within the range from 60 to 80°C;

(3) pH Stability within the range from 4.5 to 10.0; and

(4) Thermostability which allow 90% or more of enzymatic activity to remain even after exposure at 80°C for 6 hours.

5. The novel transferase claimed in any one of Claims 1 to 4, wherein the isoelectric point measured by isoelectric focusing is pH 5.3 to pH 6.3.

6. The novel transferase claimed in any one of Claims 1 to 5, wherein its activity can be fully inhibited with 5 mM $CuSO_4$.

7. The novel transferase claimed in any one of Claims 1 to 6, wherein the transferase is derived from an archaebacterium belonging to the order *Sulfolobales*.

8. The novel transferase claimed in Claim 7, wherein the transferase is derived from an archaebacterium belonging to the genus *Sulfolobus*.

9. The novel transferase claimed in Claim 7, wherein the transferase is derived from an archaebacterium belonging to the genus *Acidianus*.

10. The novel transferase claimed in Claim 8, wherein the archaebacterium belonging to the genus *Sulfolobus* is the *Sulfolobus solfataricus* strain KM1 (FERM BP-4626).

11. The novel transferase claimed in Claim 8, wherein the archaebacterium belonging to the genus *Sulfolobus* is the *Sulfolobus solfataricus* strain DSM 5833.

12. The novel transferase claimed in Claim 8, wherein the archaebacterium belonging to the genus *Sulfolobus* is the *Sulfolobus acidocaldarius* strain ATCC 33909.

13. The novel transferase claimed in Claim 9, wherein the archaebacterium belonging to the genus *Acidianus* is the *Acidianus brierleyi* strain DSM 1651.

14. A process for producing the transferase which is claimed in any one of Claims 1 to 13, wherein said process comprises cultivating a bacterium having an ability of producing the transferase claimed in any one of Claims 1 to 13 in a culture medium, and isolating and purifying said transferase from the culture according to an activity-measuring method in which the index is the activity of producing a trehaloseoligosaccharide from a substrate maltooligosaccharide.

15. The process claimed in Claim 14, wherein an archaebacterium belonging to the order *Sulfolobales* is cultivated.

16. The process claimed in Claim 15, wherein an archaebacterium belonging to the genus *Sulfolobus* is cultivated.

17. The process claimed in Claim 15, wherein an archaebacterium belonging to the genus *Acidianus* is cultivated.

18. The process claimed in Claim 16, wherein the *Sulfolobus solfataricus* strain KM1 (FERM BP-4626) belonging to the genus *Sulfolobus* is cultivated.

19. The process claimed in Claim 16, wherein the *Sulfolobus solfataricus* strain DSM 5833 belonging to the genus *Sulfolobus* is cultivated.

20. The process claimed in Claim 16, wherein the *Sulfolobus acidocaldarius* strain ATCC 33909 belonging to the genus *Sulfolobus* is cultivated.

21. The process claimed in Claim 17, wherein the *Acidianus brierleyi* strain DSM 1651 belonging to the genus *Acidianus* is cultivated.

22. A process for producing a saccharide, a couple of sugar units at an end of the saccharide being α-1,α-1-linked, wherein the transferase claimed in any one of Claims 1 to 13 is used and allowed to act on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three glucose residues from the reducing end are α-1,4-linked, so as to produce a saccharide in which at least three sugar units from the reducing end side are glucose residues and the linkage between the first and second glucose residues from

the reducing end side is α-1,α-1 while the linkage between the second and third glucose residues from the reducing end side is α-1,4.

23. The process claimed in Claim 22, wherein the substrate is each or a mixture of maltooligosaccharides.

24. The process claimed in Claim 23, wherein a trehaloseoligosaccharide such as glucosyltrehalose and maltooligosyltrehalose is produced.

25. An novel amylase which acts on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three sugar units from the reducing end are glucose residues, so as to liberate principally monosaccharides and/or disaccharides by hydrolyzing the substrate saccharide from the reducing end side.

26. The novel amylase claimed in Claim 25 which has a principal activity of acting on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three sugar units from the reducing end side are glucose residues and the linkage between the first and the second glucose residues from the reducing end side is α-1,α-1 while the linkage between the second and the third glucose residues from the reducing end side is α-1,4, so as to liberate α,α-trehalose by hydrolyzing the α-1,4 linkage between the second and the third glucose residues.

27. The novel amylase claimed in Claim 25 or 26, wherein said amylase also has an activity of endotype-hydrolyzing one or more α-1,4 linkages within the molecular chain of a substrate.

28. The novel amylase claimed in Claim 25, 26 or 27,

wherein said amylase has an activity of hydrolyzing a substrate trehaloseoligosaccharide such as glucosyltrehalose and maltooligosyltrehalose at the α-1,4 linkage between the second and the third glucose residues from the reducing end side to liberate α,α-trehalose.

29. The novel amylase claimed in any one of Claims 25 to 28, wherein its molecular weight measured by SDS-polyacrylamide gel electrophoresis is 61,000 to 64,000, approximately.

30. The novel amylase claimed in any one of Claims 25 to 29, wherein the amylase has the following physical and chemical properties:

(1) Optimum pH with in the range from 4.5 to 5.5;

(2) Optimum temperature within the range from 60 to 85°C;

(3) pH Stability within the range from 4.0 to 10.0; and

(4) Thermostability which allow 100% enzymatic activity to remain even after exposure at 80°C for 6 hours.

31. The novel amylase claimed in any one of Claims 25 to 30, wherein the isoelectric point measured by isoelectric focusing is pH 4.3 to pH 5.4.

32. The novel amylase claimed in any one of Claims 25 to 31, wherein its activity can be fully inhibited with 5 mM $CuSO_4$.

33. The novel amylase claimed in any one of Claims 25 to 32, wherein the amylase is derived from an archaebacterium belonging to the order *Sulfolobales*.

34. The novel amylase claimed in Claim 33, wherein the amylase is derived from an archaebacterium belonging to the genus *Sulfolobus*.

35. The novel amylase claimed in Claim 34, wherein the archaebacterium belonging to the genus *Sulfolobus* is the *Sulfolobus solfataricus* strain KM1 (FERM BP-4626) or a variant thereof.

36. The novel amylase claimed in Claim 34, wherein the archaebacterium belonging to the genus *Sulfolobus* is the *Sulfolobus solfataricus* strain DSM 5833 or a variant thereof.

37. The novel amylase claimed in Claim 34, wherein the archaebacterium belonging to the genus *Sulfolobus* is the *Sulfolobus acidocaldarius* strain ATCC 33909 or a variant thereof.

38. A process for producing the amylase which is claimed in any one of Claims 25 to 37, wherein said process comprises cultivating a bacterium having an ability of producing the amylase claimed in any one of Claims 25 to 37 in a culture medium, and isolating and purifying said amylase from the culture according to an activity-measuring method in which the index is the activity of producing α,α-trehalose from a substrate trehaloseoligo-saccharide.

39. The process for producing amylase claimed in Claim 38, wherein an archaebacterium belonging to the order *Sulfolobales* is cultivated.

40. The process for producing amylase claimed in Claim 39, wherein an archaebacterium belonging to the genus *Sulfolobus* is cultivated.

41. The process for producing amylase claimed in Claim 40, wherein the *Sulfolobus solfataricus* strain KM1 (FERM BP-4626) belonging to the genus *Sulfolobus* is cultivated.

42. The process for producing amylase claimed in Claim 40, wherein the *Sulfolobus solfataricus* strain DSM 5833

belonging to the genus *Sulfolobus* is cultivated.

43. The process for producing amylase claimed in Claim 40, wherein the *Sulfolobus acidocaldarius* strain ATCC 33909 belonging to the genus *Sulfolobus* is cultivated.

44. A process for producing α,α-trehalose, wherein the novel amylase claimed in any one of Claim 25 to 37 is used in combination with a transferase which acts on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three glucose residues from the reducing end are α-1,4-linked, so as to transfer the first α-1,4 linkage from the reducing end into an α-1,α-1 linkage.

45. The process for producing α,α-trehalose claimed in Claim 44, wherein said amylase and said transferase are put into a reaction at 60 to 80°C.

46. The process for producing α,α-trehalose claimed in Claim 44 or 45, wherein the concentrations of said amylase and said transferase in the reaction mixture are 1.5 Units/ml or more and 0.1 Unit/ml or more, respectively.

47. The process for producing α,α-trehalose claimed in Claim 44 or 45, wherein the concentrations of said amylase and said transferase in the reaction mixture are 1.5 Units/ml or more and 1 Unit/ml or more, respectively, and the ratio of the amylase concentration to the transferase concentration is 0.075 to 100.

48. The process for producing α,α-trehalose claimed in Claim 47, wherein the concentrations of said amylase and said transferase in the reaction mixture are 15 Units/ml or more and 1 Unit/ml or more, respectively, and the ratio of the amylase concentration to the transferase concentration is 3 to 40.

49. The process for producing α,α-trehalose claimed in any one of Claims 44 to 48, wherein the substrate is a saccharide composed of at least three sugar units, and at least three glucose residues from the reducing end of the substrate saccharide are α-1,4-linked.

50. The process for producing α,α-trehalose claimed in any one of Claims 44 to 48, wherein the substrate is starch or a starch hydrolysate.

51. The process for producing α,α-trehalose claimed in Claim 50, wherein said starch hydrolysate is produced from starch by acidolysis or enzymatic hydrolysis.

52. The process for producing α,α-trehalose claimed in Claim 51, wherein said starch hydrolysate is obtained by using a debranching enzyme.

53. The process for producing α,α-trehalose claimed in Claim 52, wherein said debranching enzyme is pullulanase or isoamylase.

54. The process for producing α,α-trehalose claimed in any one of Claims 44 to 48, wherein the substrate is each or a mixture of maltooligosaccharides in which all the glucose residues are α-1,4-linked.

55. The process for producing α,α-trehalose claimed in Claim 44 or 45, wherein a debranching enzyme is further used in combination.

56. The process for producing α,α-trehalose claimed in Claim 55, wherein said debranching enzyme is pullulanase or isoamylase.

57. The process for producing α,α-trehalose claimed in Claim 56, wherein pullulanase or isoamylase is used in combination one or more times in at least any one of the

steps for producing α,α-trehalose.

58. The process for producing α,α-trehalose claimed in Claim 57, wherein pullulanase or isoamylase is used in combination one or more times in at least any one of the early steps for producing α,α-trehalose.

59. The process for producing α,α-trehalose claimed in any one of Claims 55 to 58, wherein the substrate is starch or a starch hydrolysate.

60. The process for producing α,α-trehalose claimed in Claim 59, wherein said starch hydrolysate is produced from starch by acidolysis or enzymatic hydrolysis.

61. The process for producing α,α-trehalose claimed in Claim 60, wherein said starch hydrolysate is obtained by using a debranching enzyme.

62. The process for producing α,α-trehalose claimed in Claim 61, wherein said debranching enzyme is pullulanase or isoamylase.

63. The process for producing α,α-trehalose claimed in any one of Claims 44 to 62, wherein an enzyme derived from an archaebacterium belonging to the order *Sulfolobales* is used as said transferase.

64. The process for producing α,α-trehalose claimed in Claim 63, wherein an enzyme derived from an archaebacterium belonging to the genus *Sulfolobus* is used as said transferase.

65. The process for producing α,α-trehalose claimed in Claim 63, wherein an enzyme derived from an archaebacterium belonging to the genus *Acidianus* is used as said transferase.

66. The process for producing α,α-trehalose claimed in Claim 64, wherein an enzyme derived from the *Sulfolobus solfataricus* strain KM1 (FERM BP-4626) or a variant thereof is used as said transferase.

67. The process for producing α,α-trehalose claimed in Claim 64, wherein an enzyme derived from the *Sulfolobus solfataricus* strain DSM 5833 or a variant thereof is used as said transferase.

68. The process for producing α,α-trehalose claimed in Claim 64, wherein an enzyme derived from the *Sulfolobus acidocaldarius* strain ATCC 33909 or a variant thereof is used as said transferase.

69. The process for producing α,α-trehalose claimed in Claim 65, wherein an enzyme derived from the *Acidianus brierleyi* strain DSM 1651 or a variant thereof is used as said transferase.

70. A DNA fragment comprising a DNA sequence which codes for the novel transferase claimed in Claim 1.

71. The DNA fragment claimed in Claim 70, wherein the optimum temperature for said novel transferase is 60 to 80°C.

72. The DNA fragment claimed in Claim 70 or 71 expressed by the restriction map shown in Fig. 26.

73. The DNA fragment claimed in Claim 70 or 71 expressed by the restriction map shown in Fig. 29.

74. A DNA fragment comprising a DNA sequence which codes for an amino acid sequence shown in Sequence No. 2 or an equivalent sequence thereof.

75. The DNA fragment claimed in Claim 74 comprising a

base sequence from the 335th base to the 2518th base of the base sequence shown in Sequence No. 1.

76. The DNA fragment claimed in Claim 74 comprising a base sequence from the 1st to the 2578th base of the base sequence shown in Sequence No. 1.

77. A DNA fragment comprising a DNA sequence which codes for an amino acid sequence shown in Sequence No. 4 or an equivalent sequence thereof.

78. The DNA fragment claimed in Claim 77 comprising a base sequence from the 816th base to the 2855th base of the base sequence shown in Sequence No. 3.

79. The DNA fragment claimed in Claim 77 comprising a base sequence from the 1st base to the 3467th base of the base sequence shown in Sequence No. 3.

80. The DNA fragment claimed in any one of Claims 70 to 79 derived from an archaebacterium belonging to the order *Sulfolobales*.

81. The DNA fragment claimed in Claim 80 derived from an archaebacterium belonging to the genus *Sulfolobus*.

82. The DNA fragment claimed in Claim 81 derived from the *Sulfolobus solfataricus* strain KM1.

83. The DNA fragment claimed in Claim 81 derived from the *Sulfolobus acidocaldarius* strain ATCC 33909.

84. A DNA fragment which hybridizes with the base sequence from the 335th base to the 2518th base of the base sequence shown in Sequence No. 1 or a complementary sequence thereof at 40°C under an ionic strength of 5 × SSC, and which codes for a novel transferase acting on a substrate saccharide, the substrate saccharide being

composed of at least three sugar units wherein at least three glucose residues from the reducing end are α-1,4-linked, so as to transfer the first α-1,4 linkage from the reducing end into an α-1,α-1 linkage; and a DNA fragment which codes for the amino acid sequence encoded by the foregoing DNA fragment.

85. A DNA fragment which hybridizes with the base sequence from the 1880th base to the 2257th base of the base sequence shown in Sequence No. 1 or a complementary sequence thereof at 60°C under an ionic strength of 6 × SSPE, and which codes for a novel transferase acting on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three glucose residues from the reducing end are α-1,4-linked, so as to transfer the first α-1,4 linkage from the reducing end into an α-1,α-1 linkage; and a DNA fragment which codes for the amino acid sequence encoded by the foregoing DNA fragment.

86. A polypeptide comprising an amino acid sequence shown in Sequence No. 2 or an equivalent sequence thereof.

87. A polypeptide comprising an amino acid sequence shown in Sequence No. 4 or an equivalent sequence thereof.

88. The polypeptide claimed in Claim 86 or 87 which acts on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three glucose residues from the reducing end are α-1,4-linked, so as to transfer the first α-1,4 linkage from the reducing end into an α-1,α-1 linkage.

89. The polypeptide claimed in any one of Claims 86 to 88, wherein the optimum temperature for said activity is 60 to 80°C.

90. A recombinant DNA molecule comprising a DNA

fragment claimed in any one of Claims 70 to 85.

91. The recombinant DNA molecule claimed in Claim 90, wherein said DNA fragment claimed in any one of Claims 70 to 85 is combined in a plasmid vector.

92. The recombinant DNA molecule claimed in Claim 90 or 91, wherein said molecule is the plasmid pKT22.

93. The recombinant DNA molecule claimed in Claim 90 or 91, wherein said molecule is the plasmid p9T01.

94. A host cell transformed with a recombinant DNA molecule claimed in any one of Claim 90 to 93.

95. The host cell claimed in Claim 94, wherein the host cell is a microorganism belonging to the genus *Escherichia* or *Bacillus*.

96. The host cell claimed in Claim 95, wherein the host cell is the *Escherichia coli* strain JM109.

97. A process for producing a recombinant novel transferase which acts on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three glucose residues from the reducing end are α-1,4-linked, so as to transfer the first α-1,4 linkage from the reducing end into an α-1,α-1 linkage, wherein said process comprises cultivating a host cell claimed in any one of Claims 94 to 96 to produce said recombinant novel transferase in the culture and collecting the transferase.

98. A process for producing a recombinant novel transferase which is encoded by a DNA fragment claimed in any one of Claims 70 to 85 or which contains a polypeptide claimed in any one of Claims 86 to 89, wherein said process comprises cultivating a host cell claimed in any one of

Claims 94 to 96 to produce said recombinant novel transferase in the culture and collecting the transferase.

99. A process for producing a trehaloseoligosaccharide in which at least three sugar units from the reducing end are glucose residues and the linkage between the first and second glucose residues from the reducing end side is α-1,α-1 while the linkage between the second and third glucose residues from the reducing end side is α-1,4, wherein the process comprises putting the recombinant novel transferase claimed in Claim 97 or 98 into contact with a saccharide, the saccharide being composed of at least three sugar units wherein at least three glucose residues from the reducing end are α-1,4-linked.

100. A DNA fragment comprising a DNA sequence which codes for the novel amylase claimed in Claim 25.

101. The DNA fragment claimed in Claim 100 comprising a DNA sequence which codes for the novel amylase claimed in Claim 26.

102. The DNA fragment claimed in Claim 100 or 101 comprising a DNA sequence which codes for a novel amylase having an activity of endotype-hydrolyzing one or more of α-1,4 linkages in a sugar chain.

103. The DNA fragment claimed in any one of Claims 100 to 102, wherein said novel amylase acts on a substrate trehaloseoligosaccharide so as to liberate α,α-trehalose by hydrolyzing the substrate at the α-1,4 linkage between the second and third glucose residues from the reducing end side.

104. A DNA fragment comprising a DNA sequence which codes for a novel amylase having the following principal activities:

(1) An activity of endotype-hydrolyzing one or more

of α-1,4 glucoside linkages in a sugar chain;

(2) an activity of acting on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three sugar units from the reducing end are α-1,4-linked glucose residues, so as to liberate principally monosaccharides and/or disaccharides by hydrolyzing the substrate from the reducing end side; and

(3) an activity of acting on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three sugar units from the reducing end side are glucose residues and the linkage between the first and second glucose residues from the reducing end side is α-1,α-1 while the linkage between the second and third glucose residues from the reducing end side is α-1,4, so as to liberate α,α-trehalose by hydrolyzing the α-1,4 linkage between the second and third glucose residues from the reducing end side.

105. The DNA fragment claimed in any one of Claims 100 to 104, wherein the optimum temperature for said novel amylase is 60 to 85°C.

106. The DNA fragment claimed in any one of Claims 100 to 105 expressed by the restriction map shown in Fig. 34.

107. The DNA fragment claimed in any one of Claims 100 to 105 expressed by the restriction map shown in Fig. 38.

108. A DNA fragment comprising a DNA sequence which codes for an amino acid sequence shown in Sequence No. 6 or an equivalent sequence thereof.

109. The DNA fragment claimed in Claim 108 comprising the base sequence from the 642nd base to the 2315th base of the base sequence shown in Sequence No. 5.

110. The DNA fragment claimed in Claim 108 comprising

the base sequence from the 639th base to the 2315th base of the base sequence shown in Sequence No. 5.

111. The DNA fragment claimed in Claim 108 comprising the base sequence from the 1st base to the 2691st base of the base sequence shown in Sequence No. 5.

112. A DNA fragment comprising a DNA sequence which codes for an amino acid sequence shown in Sequence No. 8 or an equivalent sequence thereof.

113. The DNA fragment claimed in Claim 112 comprising the base sequence from the 1176th base to the 2843th base of the base sequence shown in Sequence No. 7.

114. The DNA fragment claimed in Claim 112 comprising the base sequence from the 1st base to the 3600th base of the base sequence shown in Sequence No. 7.

115. The DNA fragment claimed in any one of Claims 100 to 114, wherein said DNA fragment is derived from an archaebacterium belonging to the order *Sulfolobales*.

116. The DNA fragment claimed in Claim 115, wherein said DNA fragment is derived from an archaebacterium belonging to the genus *Sulfolobus*.

117. The DNA fragment claimed in Claim 116, wherein said DNA fragment is derived from the *Sulfolobus solfataricus* strain KM1.

118. The DNA fragment claimed in Claim 116, wherein said DNA fragment is derived from the *Sulfolobus acidocaldarius* strain ATCC 33909 or a variant thereof

119. A DNA fragment which hybridizes with the base sequence from the 639th or 642nd base to the 2315th base of the base sequence shown in Sequence No. 5 or a

complementary sequence thereof at 40°C under an ionic strength of 5 × SSC, and which codes for a novel amylase having an activity of acting on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three sugar units from the reducing end are glucose residues, so as to liberate principally monosaccharides and/or disaccharides by hydrolyzing the substrate from the reducing end side; and a DNA fragment which codes for the amino acid sequence encoded by the foregoing DNA fragment.

120. A DNA fragment which hybridizes with the base sequence from the 639th or 642nd base to the 2315th base of the base sequence shown in Sequence No. 5 or a complementary sequence thereof at 40°C under an ionic strength of 5 × SSC, and which codes for a novel amylase having a principal activity of acting on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three sugar units from the reducing end side are glucose residues and the linkage between the first and second glucose residues from the reducing end side is α-1,α-1 while the linkage between the second and third glucose residues from the reducing end side is α-1,4, so as to liberate α,α-trehalose by hydrolyzing the α-1,4 linkage between the second and third glucose residues; and a DNA fragment which codes for the amino acid sequence encoded by the foregoing DNA fragment.

121. A DNA fragment which hybridizes with the base sequence from the 1393th base to the 2121th base of the base sequence shown in Sequence No. 7 or a complementary sequence thereof at 60°C under an ionic strength of 6 × SSPE, and which codes for a novel amylase having an activity of acting on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three sugar units from the reducing end are glucose residues, so as to liberate principally monosaccharides and/or disaccharides by hydrolyzing the

substrate from the reducing end side; and a DNA fragment which codes for the amino acid sequence encoded by the foregoing DNA fragment.

122. A DNA fragment which hybridizes with the base sequence from the 1393th base to the 2121th base of the base sequence shown in Sequence No. 7 or a complementary sequence thereof at 40°C under an ionic strength of 6 × SSPE, and which codes for a novel amylase having a principal activity of acting on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three sugar units from the reducing end side are glucose residues and the linkage between the first and second glucose residues from the reducing end side is α-1,α-1 while the linkage between the second and third glucose residues from the reducing end side is α-1,4, so as to liberate α,α-trehalose by hydrolyzing the α-1,4 linkage between the second and third glucose residues; and a DNA fragment which codes for the amino acid sequence encoded by the foregoing DNA fragment.

123. A polypeptide comprising an amino acid sequence shown in Sequence No. 6 or an equivalent sequence thereof.

124. A polypeptide comprising an amino acid sequence shown in Sequence No. 8 or an equivalent sequence thereof.

125. The polypeptide claimed in Claim 123 further comprising Met at the N terminus.

126. The polypeptide claimed in any one of Claims 123 to 125 which has an activity of acting on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three sugar units from the reducing end side are glucose residues and the linkage between the first and second glucose residues from the reducing end side is α-1,α-1 while the linkage between the second and third glucose residues from the reducing end

side is α-1,4, so as to liberate α,α-trehalose by hydrolyzing the α-1,4 linkage between the second and third glucose residues.

127. The polypeptide claimed in any one of Claims 123 to 125 which has the following principal activities:

(1) An activity of endotype-hydrolyzing one or more of α-1,4 glucoside linkages in a sugar chain;

(2) an activity of acting on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three sugar units from the reducing end are α-1,4-linked glucose residues, so as to liberate principally monosaccharide and/or disaccharide by hydrolyzing the substrate from the reducing end side; and

(3) an activity of acting on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three sugar units from the reducing end side are glucose residues and the linkage between the first and second glucose residues from the reducing end side is α-1,α-1 while the linkage between the second and third glucose residues from the reducing end side is α-1,4, so as to liberate α,α-trehalose by hydrolyzing the α-1,4 linkage between the second and third glucose residues.

128. The polypeptide claimed in any one of Claims 123 to 127, wherein the optimum temperature for its action is 60 to 85°C.

129. A recombinant DNA molecule comprising a DNA fragment claimed in any one of Claims 100 to 122.

130. The recombinant DNA molecule claimed in Claim 129, wherein said DNA fragment claimed in any one of Claims 100 to 122 is combined in a plasmid vector.

131. The recombinant DNA molecule claimed in Claim 129

or 130, wherein said molecule is the plasmid pKA2.

132. The recombinant DNA molecule claimed in Claim 129 or 130, wherein said molecule is the plasmid p09A1.

133. A host cell transformed with a recombinant DNA molecule claimed in any one of Claim 129 to 132.

134. The host cell claimed in Claim 133, wherein the host cell is a microorganism belonging to the genus *Escherichia* or *Bacillus*.

135. The host cell claimed in Claim 134, wherein the host cell is the *Escherichia coli* strain JM109.

136. A process for producing a recombinant novel amylase which has a principal activity of acting on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three sugar units from the reducing end side are glucose residues and the linkage between the first and second glucose residues from the reducing end side is α-1,α-1 while the linkage between the second and third glucose residues from the reducing end side is α-1,4, so as to liberate α,α-trehalose by hydrolyzing the α-1,4 linkage between the second and third glucose residues, wherein said process comprises cultivating a host cell claimed in any one of Claims 133 to 135 to produce said recombinant novel amylase in the culture, and collecting the amylase.

137. A process for producing a recombinant novel amylase which is encoded by a DNA fragment claimed in any one of Claims 100 to 122 or which contains a polypeptide claimed in any one of Claims 123 to 128, wherein said process comprises cultivating a host cell claimed in any one of Claims 133 to 135 to produce said recombinant novel amylase in the culture, and collecting the amylase.

138. A process for producing α,α-trehalose, wherein the process comprises putting the novel transferase claimed in any one of Claim 1 to 13, or the recombinant novel transferase claimed in Claim 97 or 98, and the recombinant novel amylase claimed in Claim 136 or 137 into contact with a saccharide, the saccharide being composed of at least three sugar units wherein at least three glucose residues from the reducing end are α-1,4-linked.

139. A process for producing α,α-trehalose, wherein the process comprises putting the recombinant novel transferase claimed in Claim 97 or 98, and the novel amylase claimed in any one of Claim 25 to 37, or the recombinant novel amylase claimed in Claim 136 or 137 into contact with a saccharide, the saccharide being composed of at least three sugar units wherein at least three glucose residues from the reducing end are α-1,4-linked.

140. The process claimed in Claim 138 or 139, wherein the saccharide, which is composed of at least three sugar units wherein at least three glucose residues from the reducing end are α-1,4-linked, is starch or a starch hydrolysate.

141. The process claimed in Claim 140, wherein said starch hydrolysate is produced from starch by acidolysis or enzymatic hydrolysis.

142. The process claimed in Claim 140, wherein said starch hydrolysate is produced by hydrolyzing starch with a debranching enzyme.

143. The process claimed in Claim 142, wherein said debranching enzyme is pullulanase or isoamylase.

144. The process claimed in Claim 138 or 139, wherein the saccharide, which is composed of at least three sugar units wherein at least three glucose residues from the

reducing end are α-1,4-linked, is each or a mixture of maltooligosaccharides in which all the glucose residues are α-1,4-linked.

145. The process claimed in any one of Claims 138 to 144, wherein said process is performed at a temperature of 50 to 85°C.